the RP concentration in the cytosol. The marked increase of concentration of RP in fibromyoma cells is in agreement with the estrogen sensitivity of these tumoral cells.

Even though the concentration of steroid receptors in human tissue vary markedly according to the patient, the estrogen (RE) and progesterone (RP) receptors can be compared in two tissues either normal or tumoral in so far as they are collected from the same hysterectomized patient. Fibromyoma and myometrium were separately homogenized in Tris–EDTA–thioglycerol buffer, pH 7.4. Cytosol and KCl (0.5 M) nuclear extracts were prepared, and then incubated *in vitro* with [^{3}H]-estradiol or [^{3}H]-R_{5020} in order to determine specific binding at 0–2°C by Dextran-coated-charcoal assay or glycerol gradient analysis. In 13 cases, no qualitative differences were observed for RE and RP between the normal and the tumoral tissues. The dissociation constants of the accessible cytosol receptors were 0.21 ± 0.09 nM for RE and 1.2 ± 0.4 nM for RP. RE and RP migrated in a glycerol gradient as specific 7–8*S* and 4*S* peaks. The binding of [^{3}H]-R_{5020} was inhibited by progesterone but not by E_2, tamoxifen, or dexamethasone. The properties of the nuclear exchanged RP were similar in the myometrium and fibromyoma. A testosterone binding protein ($K_D = 0.6$ nM, 10 fmol per mg protein) was found in both tissues, and characterized in order to discriminate between the androgen receptor and the sex steroid binding plasma protein.

8. Evidence for binding of progestins to progesterone receptor in the human uterus, KESHO R. LAUMAS and ATTAN KASID, Department of Reproductive Biology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110016, India

Evidence has been presented to demonstrate that in the human uterine cytosol, progestins like norethindrone acetate (ENTA) and norethindrone (ENT) bind (to a varying degree) to the same binding unit of the receptor as that for progesterone (P). The double reciprocal plots indicated that ENTA and ENT were competitive inhibitors of progesterone for P-receptor. However, ENT affinity for P-receptor ($K_i \sim 6.8 \times 10^{-9}$ M) was comparable to progesterone, whereas ENTA competed with an affinity ($K_i \sim 7.2 \times 10^{-8}$ M), about 9-fold less than progesterone. Like P-receptor in the human uterus, ENTA/ENT binding proteins possessed the following molecular characteristics: mean *S* value 4.2; molecular weight 72000; Stokes radius 39 Å; relative mobility (R_F) 0.48 in 7.5% acrylamide gels; mean molecular radius 2.71 nm and isoelectric point 4.6. The number of specific binding sites for P, ENT and ENTA ranged from 2000 to 7000 sites/cell with K_D values of $2.5–6.5 \times 10^{-10}$ M for P and ENT and 2.1×10^{-9} M for ENTA. The rate of dissociation was slow for the cytosol ENTA-receptor complex ($t_{1/2} \sim 90$ min) as compared with cytosol P-receptor complex ($t_{1/2} \sim 55$ min) and furthermore receptor ENTA complex dissociated more slowly ($t_{1/2} \sim 5.5$ h) from the chromatin than did the receptor-P complex ($t_{1/2} \sim 3$ h). Thus it may be concluded that (1) progestins like ENTA/ENT and progesterone bind (with different affinities) to the same locus/loci on the receptor molecule and their action *via* a common receptor is conceivable; (2) the high progestational activity of ENTA (in spite of its low receptor binding) mainly depends on its slow rate of dissociation from the receptor.

9. Estradiol and estrone "receptors" in human endometrium during the menstrual cycle, FRANCIS BAYARD, CHRISTINE MOURE, SUZANNE JOSIAN, BRIGITTE KREITMANN et BERNADETTE DERACHE, Laboratoire de'Endocrinologie Expérimentale, Université Paul Sabatier, et Laboratoire de Génétique Cellulaire, Institut National de la Recherche Agronomique, Toulouse 31077, France

It has been previously shown that the total estrogen "receptor" concentration and the enzymatic activity of the estradiol-17β dehydrogenase varied in a reciprocal fashion in the human endometrium during the normal menstrual cycle: high "receptor" concentration and low enzyme activity during the follicular phase and vice versa during the luteal phase. We have studied the binding characteristics of estradiol (E_2) and estrone (E_1) to the endometrial estrogen "receptors" in order to define the biological importance of this oxidation reaction. It was observed that the estrogen "receptors" are heterogenous. Scatchard analysis of [^{3}H]-E_2 and [^{3}H]-E_1 binding at 4°C to endometrial cytoplasmic extracts revealed an apparent K_D of 1.7×10^{-10} for E_2 and of 1.9×10^{-10} M for E_1, and a number of binding sites different for E_2 (RE_2) and for E_1 (RE_1). The ratio RE_1/RE_2 was different with the date of the endometrial biopsy and a positive linear relationship was observed between this ratio and the plasma P concentration ($r = 0.72$, $P < 0.005$, $n = 16$). Similar results have been obtained in a breast cancer cell line in culture (MCF-7). In these cells, different biological activities of E_2 and E_1 added to a steroid free medium could be demonstrated, E_2 stimulating but E_1 inhibiting the cell growth. Although the respective biological activities of E_2 and E_1 in the endometrium cannot be drawn from these culture experiments, the results obtained suggest that a different action of the two steroids on the endometrial cells should also be considered (supported by grants INSERM 76-1-491 and CNRS 2397).

NEWER TECHNIQUES IN IMMUNOENZYMOLOGY

10. A steroid immunoassay based on antibody-enhanced hydrolysis of a steroid–umbelliferone conjugate, F. KOHEN, Z. HOLLANDER, J. F. BURD and R. C. BOGUSLASKI, Weizmann Institute of Science, Rehovot, Israel, and Ames Co., Elkhart, IN, U.S.A.

A homogeneous immunoassay for 17α-OH-progesterone (17-OH-P) has been developed utilizing a 17α-hydroxyprogesterone-umbelliferone (17-OH-P-U) conjugate that yields fluorescent products upon hydrolysis. 17α-Hydroxyprogesterone-7α-carboxyethyl thioether (17-OH-P-CET) was conjugated to umbelliferone through an ester bond. The rate of hydrolysis of this conjugate was enhanced upon addition of anti-17α-OH-P-CET-BSA (anti-17-OH-P) IgG fraction to the solution. The liberated label was determined by fluorimetry. Rabbit IgG from a non-immunized rabbit had no effect on the rate of hydrolysis of 17-OH-P-U. The antibody-enhanced hydrolysis of 17-OH-P-U was reversed by the presence of free homologous ligand. A linear relationship was observed between inhibition of the hydrolysis of 17-OH-P-U and 17-OH-P within the range 0.02–20 μM. Closely related steroids such as testosterone, progesterone, cortisol and estradiol did not significantly reverse the antibody-enhanced hydrolysis of 17-OH-P-U. The assay permitted the determination of free 17α-OH progesterone at a sensitivity of 4 ng/tube, using short reaction times (50 min), without the need for physical separation of the bound and free forms of the ligand or for expensive instrumentation. Furthermore, this assay eliminated the need for enzymic hydrolysis of the ligand-fluorescent marker conjugate, thus reducing the number of reagents in the system. This method is one of the simplest techniques described so far for homogeneous immunoassay, utilizing an enzyme-like property of antibody and a ligand-fluorescent dye conjugate.